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(54) Title: A TRANSCRIPTION FACTOR TFIID SUBUNIT, TAF_{II}105, POLYPEPTIDES, DNA ENCODING THEREFOR AND PHARMACEUTICAL COMPOSITIONS

**(57) Abstract**

A DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF_{II}105 polypeptide subunit of the general transcription factor TFIID, or a modified fragment of the polypeptide. The fragment or modified fragment have a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide. Also disclosed are the polypeptide fragments, as well as pharmaceutical compositions comprising the DNA molecule or the polypeptide fragment for treatment of diseases related to the pathological occurrence of apoptosis.

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A TRANSCRIPTION FACTOR TFIID SUBUNIT, TAF_{II}105, POLYPEPTIDES, DNA ENCODING THEREFOR AND PHARMACEUTICAL COMPOSITIONS

5 FIELD OF THE INVENTION

This invention relates to novel fragments of the transcription factor subunit TAF_{II}105 and to DNA molecules encoding them, and to pharmaceutical compositions and therapeutic methods using TAF_{II}105 and fragments thereof.

BACKGROUND OF THE INVENTION

10 References referred to in the present specification are listed at the end of the specification before the claims.

Initiation of transcription is a key regulatory step affecting gene expression in response to a variety of extra- and intracellular signals, during developmental processes and for providing tissue specificity. The rate of
15 transcription initiation is determined by proximal and distal enhancer elements that are bound by gene-specific transcription factors (activators or repressors); these are modular in their nature, typically consisting of a DNA binding domain and one or more activation (or repression) domains. The transcription initiation site is determined by a number of general transcription factors (GTFs)
20 that assemble around the core promoter to form the preinitiation complex. The general transcription factor TFIID plays an essential role in transcription initiation, as it recognizes and binds the core promoter and nucleates the assembly of the other general initiation factors (TFIIA, TFIIB, TFIIE, TFIIF,

and TFIID) and RNA polymerase II (for recent reviews see Hoffman et. al., 1997 and Orphanides *et al.*, 1996).

Studies of transcriptional activation mechanisms revealed that TFIID is also required for mediating transcription-activation signals by gene-specific activators (Horikoshi *et al.*, 1988; Pugh and Tjian, 1990). TFIID is a multisubunit complex that consists of the TATA box-binding protein (TBP) and a number of TBP-associated factors (TAFs) that are present in all cells (Verrijzer and Tjian, 1996). Some of these TAFs have been shown to directly bind activation domains of activators. This interaction appears to be essential for activator-dependent transcriptional stimulation *in vitro* as revealed in reconstituted transcription reactions.

A novel TFIID subunit, TAF_{II}105, that is highly enriched in TFIID of human and mouse B-lymphocytes, has recently been identified and cloned (Dikstein *et al.*, 1996, and U.S. 5,710,025, whose contents are incorporated herein by reference). TAF_{II}105 exists in sub-stoichiometric amounts relative to the core TAFs, consistent with the assumption that a TFIID complex containing this subunit might be required for transcription of a limited set of genes. TAF_{II}105 contains two distinct functional domains: a conserved C-terminal domain implicated in TFIID binding and TAF-TAF interactions (putative amino acids 553-802), and an N-terminal coactivator domain (amino acid 1-552) that directs interaction with activation domains of transcription factors. These findings suggest that TAF_{II}105 may be a coactivator that mediates activation signals of gene specific activators.

Apoptosis (or programmed cell death) is a natural cellular mechanism for eliminating unwanted cells in an organism. Apoptosis may occur as a result of an external signal, such as the presence of a cytokine, or in response to an internal cellular signal. Pathological conditions involving apoptosis may occur in several variations. On the one hand, undesired apoptosis may occur in healthy cells, while on the other hand, apoptosis may be inhibited in diseased cells, such as cancer cells.

The NF- κ B family of transcription factors are important regulators of a large number of biological processes. For example, NF- κ B plays an essential role in a variety of immune and inflammatory responses, mediates antigen and cytokine-induced gene expression and is required for cellular response to various external signals. The cytokine TNF- α activates NF- κ B by inducing rapid nuclear translocation of NF- κ B proteins. Recent studies have revealed that NF- κ B protects cells from TNF- α induced apoptosis, presumably by activating anti-apoptotic genes (Beg and Baltimore, 1996; Wang et al., 1996; van Antwerp et al., 1996; Liu et al., 1996). Moreover, activation of anti-apoptotic genes by NF- κ B has been shown to be essential for transformation by oncogenic Ras (Finco et al., 1997; Mayo et al., 1997). Likewise, activation of NF- κ B by ionizing radiation or daunorubicin was found to protect cells from killing by apoptosis (Wang et al., 1996).

15 SUMMARY OF THE INVENTION

The present invention is based on the surprising discovery that TAF_{II}105 interacts directly with members of the NF- κ B family and mediates gene activation with respect to apoptotic cellular mechanisms. Although TAF_{II}105 was previously described, and it was suggested that TAF_{II}105 may be responsible for mediating transcription by a subset of activators in B cells, no indication was given that TAF_{II}105 might be involved with NF- κ B proteins, which function also in non-B cells, nor with the activation of anti-apoptotic genes.

It is an object of the present invention to provide novel polypeptide fragments of the TFIID polypeptide subunit, TAF_{II}105, as well as DNA molecules encoding them.

It is a further object of the invention to provide pharmaceutical compositions and methods for use within the framework of a therapy intended to eliminate undesired, pathological cells, e.g. within the framework of cancer therapy.

It is a still further object of the invention to provide pharmaceutical compositions and methods for treating diseases resulting from the pathological occurrence of apoptosis.

In the present specification, the term "*normal biological activity*" of the TAF_{II}105 polypeptide refers to mediation by TAF_{II}105 of the activation of anti-apoptotic genes by NF- κ B, as described hereinafter.

According to one aspect of the present invention, there is provided a DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2, wherein the fragment has a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide.

Such fragments may be derived from the N- or C-terminus of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2, but preferably they are derived from the N-terminal domain, which is the putative coactivator domain of TAF_{II}105. A most preferred fragment consists of the N-terminal amino acids 1 to 552 of TAF_{II}105, named herein TAF_{II}105 Δ C. Other preferred fragments contain a sequence of amino acids included within TAF_{II}105 Δ C such as the fragments consisting of the amino acids 1-452, 1-359 and 443-452, or is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids such that the thus obtained modified fragment has a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2.

Based on the discovery, in accordance with the invention, of the role that TAF_{II}105 plays in the apoptotic process, the present invention provides novel therapies allowing, on the one hand, to promote apoptosis of pathological cells and, on the other hand, to prevent undesired apoptosis in normal cells. In the first case, the apoptosis-inhibiting pathway which is mediated by TAF_{II}105 is inhibited by neutralizing, directly or indirectly, the TAF_{II}105 polypeptide, and in the second case, this apoptosis-inhibiting pathway is enhanced.

In the first aspect of the invention, referred to at times as the "*apoptosis-promoting aspect*", the active TAF_{II}105 fragments of the invention

are used to promote and potentiate the apoptotic mechanism, for example in the treatment of cancer, in which case it is desired to stimulate apoptosis in the cancerous cell. Thus, the active TAF_{II}105 fragment or a composition containing it may be administered by itself to the patient, or it may be administered together
5 with another chemotherapeutic agent so as to increase its effectiveness.

Thus, according to this aspect of the invention, there is provided a pharmaceutical composition for inducing an apoptotic process in pathological cells, for example in the treatment of cancer, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

- 10 (a) a fragment of the TAF_I105 polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or more amino acids, wherein the fragment or the modified fragment has a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide;
- 15 (b) an inhibitor or antagonist of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2;
- (c) a DNA sequence encoding the fragment of (a); and
- (d) a DNA sequence directing expression of an antisense RNA sequence to SEQ.ID. NO.: 1, or a part thereof, and which is
20 capable of inhibiting its expression *in vivo*.

Also provided according to this aspect of the invention is the use of an active agent as defined in (a) – (d) above for the preparation of a pharmaceutical composition for use for promoting apoptosis, for example in the treatment of cancer, as well as a method for the promotion of apoptosis, for
25 example in the treatment of cancer, comprising administering to a subject in need, an active agent as defined in (a) – (d) above or a composition comprising such agent.

In the second aspect of the invention, referred to at times as the “apoptosis-preventing aspect”, the active agents are used to interfere with the
30 apoptosis mechanism in situations where it is detrimental to the health of the

organism. Such situations include, for example, the undesired induction of apoptosis in autoimmune diseases, inflammatory processes and viral or bacterial infections.

Thus, according to this aspect, the present invention provides a pharmaceutical composition for use in the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:

- (e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;
- 10 (f) a DNA molecule consisting of a DNA sequence encoding the TAF_{II}105 polypeptide of SEQ. ID. NO: 2;
- (g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the TAF_{II}105 polypeptide of
- 15 SEQ. ID. NO: 2;
- (h) the TAF_{II}105 polypeptide of SEQ. ID. NO: 2; and
- (i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified
- 20 polypeptide retains the normal biological activity of the TAF_{II}105 polypeptide encoded by SEQ. ID. NO: 2.

Also provided according to this aspect of the invention is the use of an active agent as defined in (e) – (i) above for the preparation of a pharmaceutical composition for use in the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections, as well as a method for the treatment of pathological apoptosis of cells, for example in autoimmune diseases, inflammatory processes and viral or bacterial infections,, comprising administering to a subject in need, an active agent as defined in (e) – (i) above or a composition comprising such

30 agent.

The aforementioned active agents of both aspects of the invention may be administered to an individual by the use of appropriate carriers, which may be selected from a number of such carriers known *per se*. For example, where said active agent is a DNA sequence or a modified DNA sequence, it will typically be administered within the framework of a vector, as known in the art of gene therapy. As will be appreciated, the vector, which may be a plasmid, viral particle, etc., should preferably be constructed so that it will direct the active DNA sequence into the target cells, namely, cells in which apoptosis is to be induced or cells undergoing undesired apoptosis. Furthermore, the vector should also have the necessary promoters so as to induce expression of the DNA sequence. The manner of construction of appropriate vectors including ensuring appropriate targeting and expression control of the inserted DNA sequence, are generally known and the man of the art should have no difficulties, based on the teaching of the present invention and the general knowledge available in the art, to construct the appropriate vectors.

It is obviously also within the reach of the artisan to prepare pharmaceutical compositions where the active agent is a peptide or polypeptide. At times, it may be desired to include a targeting vehicle in the composition so as to target the polypeptide to the cells, which vehicle may, for example, be a liposome having a targeting moiety, e.g. a ligand of the receptor, on its external surface.

As is also known, the targeting of the inventive compositions to the required site within the body, may also be achieved by direct injection or infusion of the compositions into the site.

25 DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, preferred embodiments will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 is the DNA sequence encoding the TAF_{II}105 polypeptide (SEQ. ID. NO: 1);

Fig. 2 is the deduced amino acid sequence of the TAF_{II}105 polypeptide (SEQ. ID. NO: 2);

5 **Figs. 3A-3C** illustrate the interaction of hTAF_{II}105 with p65/RelA, a member of the NF- κ B family.

3A. NF- κ B members p65/RelA and p50 and unrelated OCT2 proteins were translated *in vitro* and labeled with ³⁵S-methionine using rabbit reticulocyte lysate. These proteins were used for interaction assay with
10 immobilized flag-tagged TAF_{II}105 as indicated on the top of each lane. As a control, the labeled proteins were incubated with the flag beads. The bound proteins were eluted, resolved on SDS-PAGE and autoradiographed. Input lanes represent 10% of the labeled protein used for the binding reaction. 3B. Binding reaction between ³⁵S-labeled p65/RelA and the purified N-terminal
15 fragment of TAF_{II}105 (amino acids 1-552) fused to glutathione S-transferase (GST) and bound to glutathione beads (lane 2). As a control, a similar reaction was performed using the same beads bound by GST (lane 3). Input lanes represent 10% of p65 used for the binding assay. 3C. Interaction between
20 TAF_{II}105 and the C-terminal activation domain of p65. The C-terminus of p65 (amino acids 398-551) was expressed as a GST- fusion protein, affinity-purified by glutathione beads and subjected to a binding reaction with *in vitro* translated and ³⁵S-labeled TAF_{II}105 (lanes 1-3) or TAF_{II}130 (lanes 4-6). GST-containing beads were used as control in a similar binding reaction. Input lanes represent approximately 10% of the labeled proteins used.

25 **Figs. 4A & 4B** illustrate the interaction of additional NF- κ B rel proteins with TAF_{II}105.

4A. *In vitro* translated and ³⁵S-labeled RelB (lanes 1-3) and c-rel (lanes 4-6) were used for an *in vitro* binding assay with the N-terminus of TAF_{II}105 similar to Fig. 3 B. 4B. The C-termini of human c-rel (amino acids 302-614)

and mouse relB (amino acids 348-558) were expressed as fusion with GST protein, purified by glutathione beads and used for binding assay with *in vitro* translated and ^{35}S -labeled TAF $_{\text{II}}$ 105 (lanes 1-4) or TAF $_{\text{II}}$ 130 (lanes 5-8). Input lanes represent 10% of the labeled proteins used in the assay.

5 **Figs. 5A-5D** illustrate the stimulation of p65 and TNF- α induced NF- κ B transcriptional activity by TAF $_{\text{II}}$ 105.

5A. Human 293A cells (in 24-well plate) were cotransfected with NF- κ B-dependent luciferase reporter plasmid (50 ng) together with either an empty expression vector or the following expression plasmids: 500 ng of
10 TAF $_{\text{II}}$ 105 (columns 2, 4, 7, 8 and 10); 12.5 ng of p65 (columns 5 and 7); 25 ng of p65 (columns 6 and 8) and 500 ng of I κ B α (columns 3, 4, 9 and 10). The amount of CMV-derived vector in each transfection assay was kept constant.

5B. Similar transfection experiment using HeLa cells. In this experiment the amount of DNA used was 10-fold higher, keeping the relative amount of each
15 plasmid similar. 5C. An NF- κ B luciferase reporter plasmid was cotransfected into 293A cells using similar transfection conditions as in A, with either an empty expression vector (columns 1, 3, 7), a TAF $_{\text{II}}$ 105 expression plasmid (columns 2,5,6, and 8) or with a constitutively active I κ B α (lanes 7 and 8). 12 hours post-transfection, 0.15 ng/ml (columns 3 and 4) or 1.5 ng/ml (columns
20 5-8) of TNF- α were provided to the cells, and 24 hours after transfection, luciferase activity was determined. D. Similar transfection experiment in HeLa cells using 0.15 ng/ml TNF- α .

Figs. 6A-6C illustrate the inhibition of NF- κ B transcription by dominant negative mutant of TAF $_{\text{II}}$ 105 (TAF $_{\text{II}}$ 105 Δ C).

25 **6A.** Schematic representation of TAF $_{\text{II}}$ 105 putative functional domains and the dominant negative mutant of TAF $_{\text{II}}$ 105 (TAF $_{\text{II}}$ 105 Δ C). NLS stands for nuclear localization signal. Expression of mutated TAF $_{\text{II}}$ 105 was confirmed by Western blot (data not shown). **6B.** Luciferase reporter plasmids driven by two tandem NF- κ B sites (lanes 1-3), two mutated NF- κ B sites (lanes 4 and 5) or a
30 CMV enhancer (lanes 6 and 7) were transfected into 293A cells together with

the following plasmids: empty expression vector (lanes 1, 4 and 6), with TAF_{II}105 Δ C expression plasmid (lanes 2, 5 and 7) or dominant negative mutant of I κ B α (lane 3). **6C.** An NF- κ B reporter plasmid (50 ng) was transfected into 293A cells together with an empty expression vector (lane 1),
5 25 ng of p65/RelA expression plasmid (lanes 2-4) and increasing amounts of TAF_{II}105 Δ C expression plasmid (100 and 250 ng, lanes 3 and 4, respectively).

Figs. 7A-7C illustrate how a dominant negative mutant of TAF_{II}105 induces cell death in response to TNF- α .

7A. 293T cells were transfected with wild-type TAF_{II}105 (pictures 1
10 and 2) or TAF_{II}105 Δ C (3 and 4), and either untreated (1 and 3) or treated with 15 ng/ml TNF- α (2 and 4). The pictures were taken 24 hours after transfection.

7B. Genomic DNA analysis of 293T cells that were transfected with either an empty expression vector (lanes 1 and 2), TAF_{II}105 Δ C (lanes 3 and 4) or with wild-type TAF_{II}105 (lanes 5 and 6). Twelve hours after transfection some of
15 the transfected cells were treated with TNF- α (odd number lanes), and 24 hours after transfection DNA was extracted from the transfected cells and analyzed by 1.8% agarose gel. M stands for a 1 kB DNA size marker. **7C.** Cell survival assay. To quantify the apoptosis induction, 293T cells were cotransfected with a pCMVlacZ reporter plasmid and the indicated expression vectors, and
20 similar to the experiment described in (B), were treated with TNF- α (lanes 2, 4 and 6). 24 hours after transfection the cells were stained with X-Gal, and the numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

Figs. 8A-8C illustrate the effect of TAF_{II}105 anti-sense RNA expression
25 on TNF- α treated cells.

8A. 293T cells were transfected either with empty vector or with expression vector for TAF_{II}105 anti-sense RNA. Thirty-six hours after transfection the levels of endogenous TAF_{II}105 were analyzed by Western blot using affinity-purified anti-TAF_{II}105 antibodies (upper panel). As a control the
30 same extracts were used for analysis of TBP levels (lower panel). **8B.**

Cell-survival analysis of cells expressing TAF_{II}105 anti-sense RNA. 293T cells were cotransfected with CMV-GFP reporter plasmid with either an empty expression vector (lanes 1 and 2) or with expression vector for TAF_{II}105 anti-sense RNA (lanes 3 and 4). Twenty-four hours after transfection TNF- α was provided to the cells (lanes 2 and 4) and 24 hours later green fluorescent cells were counted in five randomly chosen fields. These data represent an average of four independent transfection experiments. **8C.** DNA fragmentation analysis of cells transfected with either anti-sense TAF_{II}105 RNA (lanes 1 and 2) or empty vector (lanes 3 and 4). Twenty-four hours after transfection TNF- α was provided to the cells (lanes 1 and 3) and 16 hours later fresh medium containing TNF- α was provided to the cells. Genomic DNA was extracted 48 hours after transfection and analyzed by 1.8% agarose gel. M represents a 1 kB DNA molecular size marker.

Figs. 9A-9C illustrate how a dominant negative mutant of TAF_{II}105 inhibits activation of anti-apoptotic genes by NF- κ B.

9A. 293T cells were transfected with the indicated expression plasmids. 24-hours after transfection, DNA was extracted from the cells and analyzed by 1.8% agarose gel. **9B.** A representative experiment as described in A. The pictures were taken 24 hours after transfection. 293T cells transfected with p65 are shown in panel 1; cells cotransfected with wild-type TAF_{II}105 and p65 are shown in panel 2; and cells cotransfected with TAF_{II}105 Δ C and p65 are shown in panel 3. Pictures of cells transfected with either TAF_{II}105 or TAF_{II}105 Δ C alone are shown in Fig. 7. **9C.** 293T cells were cotransfected with pCMVlacZ reporter plasmid and expression vectors as indicated in the figure. Twenty-four hours after transfection the cells were stained with X-Gal. The numbers of blue cells in five randomly chosen fields were determined. These data represent an average of 3 independent transfection experiments.

Figs. 10A & 10B schematically illustrate a model for the role of TAF_{II}105 (TFIID) in activation of TNF- α /NF- κ B induced anti-apoptotic genes in 293 cells;

Fig. 11 is a schematic representation of various deletion mutants of the TAF_{II}105 N-terminal domain: amino acid 1-552, 1-452, 1-359, 1-167 and 443-452, and their ability to bind p65 and their effect on the survival of p65 expressing cells.

- 5** **Fig. 12A** shows the structure of a TAF_{II}105 Δ C transgene. The DNA used to generate TAF_{II}105 Δ C transgenic animals contains a cDNA fragment encoding for the N-terminus (amino acid 1-552) of TAF_{II}105, an in frame nuclear localization signal (NLS) and influenza virus hemagglutinin tag (HA). **12B.** Southern blot showing the integration of the transgene into the genome of
- 10** mice. Restriction enzyme-digested tail DNA of several founders was analyzed for the integration of TAF_{II}105 Δ C by Southern blot using TAF_{II}105 Δ C as a probe. PC is the positive control.

DETAILED DESCRIPTION OF THE INVENTION

MATERIALS AND METHODS

15 TAF_{II}105

TAF_{II}105 was prepared as previously described (Dikstein *et al.*, 1996). The DNA and amino acid sequences are shown in Figs. 1 and 2, respectively.

20 In vitro binding experiments

Baculovirus expression of flag-tagged TAF_{II}105 was carried out as previously described (Dikstein *et al.*, 1996). ³⁵S-labeled p65, OCT2, and p50 were synthesized *in vitro* by T7 RNA polymerase and rabbit reticulocytes lysate and incubated with flag beads or with TAF_{II}105 coupled to flag beads in 0.1M

25 KCl HEMG buffer (20 mM Hepes pH 7.9, 100 mM KCl, 12.5 mM MgCl₂, 0.2 mM EDTA, 0.1% NP-40, 1mM DTT, 0.2mM PMSF) for 2 hours at 4°C. The beads were washed 3 times with the same buffer and 2 times with 0.2M NaCl HEMG buffer. The bound proteins were eluted by 5 minutes boiling in protein sample buffer followed by SDS-PAGE and autoradiography. The c-rel protein

was translated *in vitro* using T7 RNA polymerase. RelB was synthesized by T3 RNA polymerase. Binding reactions with GST-bound proteins were done in a similar way.

5 Plasmids

The baculovirus expression vector for TAF_{II}105 was previously described (Dikstein *et al.*, 1996). CMV-TAF_{II}105 was constructed by inserting a NcoI-BglII (NcoI was filled in by the Klenow enzyme) fragment containing TAF_{II}105 cDNA and HA Tag from pVLHA- TAF_{II}105 (Dikstein *et al.*, 1996) into SmaI-BamHI site of the pCGN vector (Tanaka and Herr, 1990).

To generate the TAF_{II}105 Δ C expression vector, an N-terminal fragment of TAF_{II}105 (amino acid 1-552, Dikstein *et al.*, 1996) that was generated by PCR together with an HA tag was first cloned in a CMV-nuc vector (a gift from Dr. Paz Einat) in frame with a nuclear localization signal (NLS). Next, a PstI-HindIII fragment containing NLS, HA and TAF_{II}105 Δ C was inserted into PstI-HindIII sites BlueScript KS+ vector. This fragment was then removed by SmaI and HincII and cloned in a SmaI site of pCGN. The vector for directing anti-sense TAF_{II}105 RNA expression contains the same insert, albeit in opposite orientation. Expression of both wildtype and mutant TAF_{II}105 after transient transfection was verified by Western blot using anti-HA antibodies (data not shown).

GST- TAF_{II}105 Δ C was generated by cloning an NdeI-EcoRI fragment from pET- TAF_{II}105 Δ C (Dikstein *et al.*, 1996) into NdeI-EcoRI of pGEX-2TKN. The pT β STOP-c-rel that was used for *in vitro* translation of c-rel protein was constructed by insertion of an NcoI-EcoRI fragment containing c-rel cDNA (a gift from Dr. Nancy Rice) into NcoI-EcoRI sites of the pT β STOP vector. GST-p65C was constructed by inserting an NcoI-XhoI fragment (corresponding to amino acids 398-551) from pET-p65 into NcoI-SalI of pGEX-2TKN. To generate GST-c-relC, a fragment corresponding to amino acids 302-614 was cloned into a StuI site of pGEX2TKN. GST-RelBC was

generated by inserting a BglII-BamHI fragment from pBS-RelB (amino acids 348-558, a gift from Dr. Rodrigo Bravo) into a BamHI site of pGEX-2TKN.

The NF- κ B dependent reporter plasmids (wild-type and mutant) were constructed by inserting double-stranded synthetic oligonucleotides (see below) containing either two tandem κ B sites or two mutated κ B sites next to a minimal core promoter from the mouse α -actin promoter (-40 to +80), that was cloned into Hind III site of a promotorless pLuc vector (Altschmied and Duschl 1997).

NF- κ B oligonucleotides:

10 5'-AGCTTAGGGACTTTCCGAGGGGACTTTCCG-3';
5'-GATCCGGAAAGTCCCCTCGGAAAGTCCCTA-3'

Mutated NF- κ B oligonucleotides:

5'-AGCTTATCTACTTTCCGAGTCTACTTTCCG-3';
5'-GATCCGGAAAGTAGACTCGGAAAGTAGATA-3'.

15

Propagation and Transfection of Cell Lines

293 and HeLa cell lines were maintained in F12 Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum. Transfections were performed using the standard CaPO₄ method. 293

20 subconfluent cells were transfected in a 24-well multidish using a total of 1 μ g plasmid; HeLa cells were transfected using 10 μ g of plasmid/5X10⁶ cells in 100mm petri dish. Luciferase activity was determined according to the instructions of the manufacturer (Promega).

Human recombinant TNF- α was purchased from R & D Systems.

25

Apoptosis assays

For the survival analysis, 293A or 293T cells were cotransfected with a pCMV-*lacZ* reporter plasmid and different expression vectors and stained with X-Gal (see below) 36 hr or 48 hr after transfection. The number of

30 blue cells was determined by counting five different randomly chosen fields.

For the X-Gal assay the cultures were rinsed with PBS, fixed with 1% glutaraldehyde for 15 min, and then stained with 4 ml of X-Gal solution [2 mg/ml X-Gal in DMF, 3.3 mM $K_3Fe(CN)_6$ and 3.3 mM $K_4Fe(CN)_6$] for 2 hr.

- DNA fragmentation assay was carried out as follows: 293T cells
- 5 (2X10⁶) were collected 24 hr after transfection, resuspended in 400 μ l lysis buffer (200 mM Tris pH 8.5, 100 mM EDTA, 1% SDS, and 100 μ g/ml proteinase K) and incubated overnight at 37
- extraction following ethanol precipitation. Pelleted DNA was resuspended in 100 μ l TE containing 50 μ g/ml RNase A and incubated for 30 min at 37°C.
- 10 Twenty micrograms of DNA was run on a 1.8% agarose gel in the presence of 0.5 μ g/ml ethidium bromide.

EXAMPLES

I. TAF_{II}105 interacts with the trans-activation domains of NF- κ B

15 members

- To identify TAF_{II}105 target activators, the transcription factors NF- κ B and OCT2 that are involved in B cell specific transcription were tested as to whether they could interact with TAF_{II}105. For this purpose, recombinant TAF_{II}105 was produced in Sf9 cells as a fusion with the flag epitope-tag and
- 20 purified by anti-flag antibodies coupled to agarose beads. Immobilized TAF_{II}105 was incubated with *in vitro* translated and ³⁵S-labeled p50 and p65 (relA) subunits NF- κ B family and OCT2.

- As shown in Fig. 3A, p65 but not p50 or OCT2 specifically and efficiently interacts with TAF_{II}105. To determine whether the interaction
- 25 between p65 and TAF_{II}105 is directed by the putative N-terminal coactivator domain of TAF_{II}105, this region was expressed in *E. Coli* as a fusion with glutathione S-transferase (GST105 Δ C) and subjected to a binding reaction with ³⁵S-labeled p65. As shown in Fig. 3B, p65 specifically binds to the N-terminus of TAF_{II}105. We also tested other members of the NF- κ B family (c-rel and

RelB) for interaction with TAF_{II}105. c-rel but not RelB binds to TAF_{II}105N in this assay (Fig. 4A).

Previous studies of activator-TAF association established that an interaction between coactivator subunits of TFIID and transcriptional activators is directed by the trans-activation domain of the activator (Verrijzer and Tjian, 1996). Both p65 and c-rel contain a strong activation domain located at the carboxy-terminus (reviewed by Schmitz and Baeuerle, 1995). To determine the involvement of these activation domains in TAF_{II}105 binding, we constructed plasmids for expression of the p65, c-rel and RelB C-termini as fusion with glutathione S-transferase (GST-p65C, GST-RelBC and GST-c-relC). These proteins were purified and immobilized on glutathione Sepharose beads and used in binding assays with an *in vitro* translated and ³⁵S-labeled TAF_{II}105. To determine the specificity of the interaction, a similar binding reaction was also performed with h TAF_{II}130, a highly related homologue of TAF_{II}105 (Dikstein *et al.*, 1996). The C-terminal activation domains of both p65 and c-rel specifically bind TAF_{II}105 but not TAF_{II}130 (Figs. 3C and 4B). In this assay, the activation domain of RelB weakly interacts with TAF_{II}105 (Fig. 4B).

II. NF- κ B dependent transcription is stimulated by TAF_{II}105

To determine the functional importance of TAF_{II}105-NF- κ B interaction, we tested the effect of TAF_{II}105 on transcriptional activation by p65 in transient transfection experiments. A reporter plasmid containing two NF- κ B elements upstream to a minimal core promoter and a luciferase gene was cotransfected with p65 and TAF_{II}105 into 293 cells. As shown in Fig. 5A, p65 activates NF- κ B reporter in a dose-dependent manner (columns 5 and 6). When TAF_{II}105 was also cotransfected, both the basal NF- κ B and p65 dependent activities were stimulated 2, 5 and 6 fold respectively (columns 2, 7 and 8). Likewise, TAF_{II}105 potentiates NF- κ B dependent transcription in HeLa cells (Fig. 5B).

To determine whether the activation by TAF_{II}105 requires the presence of NF- κ B proteins, a similar experiment was carried out in the presence of I κ B α , a specific inhibitor of NF- κ B factors that prevents the translocation of NF- κ B into the nucleus (reviewed in Verma *et al.*, 1995; Baldwin, 1996; Baeuerle and Baltimore, 1996). As expected, I κ B α inhibits both constitutive and p65 induced NF- κ B activity (Fig. 5A, columns 3 and 9 respectively). In the presence of I κ B, no induction of luciferase activity by TAF_{II}105 is observed (Fig. 5A, columns 4 and 10).

NF- κ B activity is regulated by a broad range of cytokines and external stimuli. TNF- α is among the physiological inducers of NF- κ B transcriptional activation. To test whether TAF_{II}105 can stimulate TNF- α induced genes, the NF- κ B reporter plasmid was transfected into 293 and HeLa cells in the presence or absence of a TAF_{II}105 expression plasmid. When the cells are stimulated with sub-optimal doses of TNF- α (0.15 and 1.5 ng/ml), TAF_{II}105 stimulates the NF- κ B dependent reporter in response to TNF- α (Figs. 5C and 5D). Here again, in the presence of I κ B no induction of NF- κ B reporter by TAF_{II}105 is observed (Fig. 5C, columns 7 and 8). Interestingly, when optimal doses of TNF- α are provided to the cells (10-15 ng/ml), or when the cells are transfected with high amount of p65 expression plasmid, cotransfected TAF_{II}105 has little or no additional effect on the NF- κ B dependent reporter plasmid that is stimulated to its maximal potential (data not shown) possibly because the basal level of TAF_{II}105 present in the cells is sufficient for transcriptional activation when NF- κ B concentration within the nucleus is high. These experiments are representative of 3-5 independent transfection experiments with similar results. Expression of transfected TAF_{II}105 was verified by Western blot (data not shown).

To further confirm that TAF_{II}105 is involved in NF- κ B activity a eukaryotic expression vector for a dominant negative mutant of TAF_{II}105 was constructed based on its predicted function domains (Dikstein *et al.*, 1996). This mutant is a truncated form of h TAF_{II}105 (TAF_{II}105 Δ C, amino acids 1-552,

Fig. 6A), containing the NF- κ B binding region but not the TFIID interaction region. Therefore, if TAF_{II}105 Δ C binds NF- κ B *in vivo*, it is likely to inhibit NF- κ B dependent transcription.

To test the effect of TAF_{II}105 Δ C on NF- κ B activity, the NF- κ B dependent reporter plasmid was cotransfected with TAF_{II}105 Δ C into 293 cells. The constitutive basal activity of NF- κ B was strongly inhibited by TAF_{II}105 Δ C (Fig. 6B). Similarly, cotransfection of the NF- κ B reporter with a dominant negative I κ B- α inhibited this basal NF- κ B activity, confirming that the observed activity is directed by NF- κ B proteins (column 3). Reporter plasmids containing either mutation within the NF- κ B sites (columns 4 and 5) or the CMV enhancer (columns 6 and 7) were not affected by TAF_{II}105 Δ C further supporting the idea that TAF_{II}105 Δ C is specific to NF- κ B and has no effect on core promoter activity.

It was next examined whether a dominant negative mutant of TAF_{II}105 can interfere with the trans-activation potential of NF- κ B proteins. 293 cells were cotransfected with NF- κ B dependent reporter plasmid and p65/RelA transcription factor together with TAF_{II}105 Δ C. As shown in Fig. 6C, TAF_{II}105 Δ C inhibited transcriptional activation by p65/RelA in a dose dependent manner. It is important to note that the amounts of TAF_{II}105 Δ C expression plasmid used in this experiment are insufficient to affect cell viability when cotransfected with p65 (see Fig. 10 below), as evident by microscopic examination and X-gal staining of transfected cells (data not shown).

III. Inhibition of TAF_{II}105 function causes apoptosis in response to TNF- α

Recent studies have revealed that activation of NF- κ B by TNF- α plays an essential role in protecting cells from pro-apoptotic stimuli produced by TNF- α (Beg and Baltimore, 1996; Wang *et al.*, 1996; Van Antwerp *et al.*, 1996; Liu *et al.*, 1996). Interestingly, when the effect of the TAF_{II}105 dominant

negative mutant on long term TNF- α stimulation in 293 cells was analyzed, we observed dramatic changes in 293 cell morphology that are consistent with cell death (Fig. 7A). These morphological changes were not observed in cells transfected with vector alone (data not shown) or with wild type TAF $_{II}$ 105 (Fig. 7A). The survival rate after TNF- α stimulation in TAF $_{II}$ 105 Δ C expressing cells was less than 5% as determined by X-gal staining of transfected cells (Fig. 7C).

To determine whether these changes are related to programmed cell death, 293 cells transiently expressing TAF $_{II}$ 105 Δ C were induced by TNF- α and assayed for the DNA ladder characteristic to apoptotic response (Fig. 7B). As expected, no DNA fragmentation appears in TNF- α induced 293 cells transfected with empty expression vector (lane 1). The DNA ladder can be clearly seen only in cells expressing TAF $_{II}$ 105 Δ C and stimulated with TNF- α (lane 3), whereas no DNA fragmentation appears in these cells in the absence of TNF- α stimulation (lane 4), or in cells expressing wild type TAF $_{II}$ 105 in the presence or absence of TNF- α stimulus (lanes 5 and 6).

To further confirm the involvement of TAF $_{II}$ 105 in activation of anti-apoptotic genes by TNF- α , 293 cells were transiently transfected with expression vector that directs expression of anti-sense TAF $_{II}$ 105 RNA. As shown in Fig. 8A, transient expression of anti-sense TAF $_{II}$ 105 reduced the endogenous level of TAF $_{II}$ 105 protein but not TBP. This reduction is significant considering that although high transfection efficiencies are achieved in these cells, there is still a significant proportion of untransfected or poorly transfected cells that express normal amounts of TAF $_{II}$ 105 (approximately 20% as determined by X-gal staining after transfection, data not shown). The reduction in TAF $_{II}$ 105 level is correlated with an increase in cell death in response to TNF- α , as evident by cell survival assay (Fig. 8B), by DNA fragmentation (Fig. 8C) and microscopic examination (data not shown).

IV. TAF $_{II}$ 105 is involved in activation of anti-apoptotic genes by NF- κ B

In addition to the role that NF- κ B plays in activation of anti-apoptotic genes, numerous reports implicated a role for NF- κ B in activation of pro-apoptotic signals (Lee *et al.*, 1995; Lin *et al.*, 1995; Grimm *et al.*, 1996). In 293 cells the involvement of NF- κ B proteins in both processes
5 has been documented (Wong *et al.*, 1989; Grimm *et al.*, 1996). When 293 cells are transfected with p65, the cells stay healthy and do not die by apoptosis (Figs. 9A, B and C), presumably because of equilibrium between pro- and anti-apoptotic signals produced by p65. Also, cotransfection of I κ B and p65 has no effect on cell survival (Fig. 9A), since I κ B prevents NF- κ B from entering the
10 nucleus and therefore inhibits any transcriptional activity by p65. Surprisingly, cotransfection of p65 with the dominant negative mutant of TAF $_{II}$ 105 resulted in a strong apoptotic response as evident by DNA fragmentation, the characteristic morphological changes, and surviving cell number (Fig. 9A, B and C respectively). By contrast, no effect is observed with cotransfection of
15 p65 and wild type TAF $_{II}$ 105 (Figs. 9A and B) or by expression of each of these factors alone (Fig. 7). These results confirm the direct role of p65 in activation of cell death in 293 cells and suggest that the TAF $_{II}$ 105 mutant inhibits activation of anti-apoptotic genes but not pro-apoptotic genes by p65. Therefore, it is likely that TAF $_{II}$ 105 is required only for activation of
20 anti-apoptotic genes by NF- κ B.

A proposal outlining TAF $_{II}$ 105 activity is summarized in Figs. 10A and 10B. TNF- α activates both anti- and pro-apoptotic cascades (Fig. 10A). The anti-apoptotic pathway requires I κ B phosphorylation and degradation followed by NF- κ B nuclear localization. In the nucleus, a complex that is
25 formed between NF- κ B and TFIID containing TAF $_{II}$ 105 selectively promotes transcription of genes that antagonize the protein synthesis-independent cytotoxic pathway induced by TNF- α . Various agents that can induce NF- κ B are also known to stimulate apoptotic signals in cells, such as H $_2$ O $_2$ derived radicals, UV-irradiation, viruses, etc. In 293 cells, activation of NF- κ B causes
30 transcriptional activation of both genes that promote apoptosis as well as genes

that inhibit it. The NF- κ B-TAF_{II}105-TFIID complex is selectively engaged in activation of anti-apoptotic genes by NF- κ B, while the mechanism of activation of pro-apoptotic genes by NF- κ B remains obscure (Fig. 10B).

5 V. **Preparation of dominant-negative TAF_{II}105 deletion mutants**

On the basis of the results described above, it is possible to inhibit specific functions of native TAF_{II}105 in a dominant negative manner by using small fragments of the TAF_{II}105 N-terminus corresponding to different activator binding sites. For example, mapping of NF- κ B binding region within TAF_{II}105 can provide
10 more efficient and specific inhibitors of NF- κ B dependent activation of anti-apoptotic genes. In order to prepare such fragments, it is possible to generate deletion mutants of the TAF_{II}105 N-terminus. These mutants may be first expressed in *E. Coli* as fusion proteins such as with glutathione S-transferase, and are then used in *in vitro* binding assays with different TAF_{II}105 target activators such as NF- κ B members.

15 To further investigate the involvement of TAF_{II}105 in NF- κ B induction of anti-apoptotic genes we generated deletion mutants of TAF_{II}105 Δ C. Various fragments from TAF_{II}105 cDNA corresponding to amino acids 1-452, 1-359, 1-167 and 443-552 were generated by restriction enzymes or by PCR. These mutants were expressed in *E. Coli*, purified and analyzed for their ability to bind p65/RelA in an
20 *in vitro* binding assay with p65/RelA and for their effect on the survival of p65 expressing cells by expressing the fragments in human 293 cells together with p65.

As shown in Fig. 11, TAF_{II}105 Δ C (1-552) potently inhibits cell survival (+++) and binds p65 with high affinity (+++). Mutants of TAF_{II}105 Δ C deleted of 100 or 193 amino acid residues [105 Δ C (1-452) and 105 Δ C (1-359)] have reduced p65
25 binding capacity (+) as well as reduced inhibitory activity (+) as compared with 105 Δ C. Similarly, a polypeptide fragment corresponding to amino acid 443-552 is capable of p65 binding and inhibits its anti-apoptotic activity (++) while a fragment (105 Δ C 1-167) that is unable to bind p65 (-) did not affect cell survival (-).

These findings indicate the existence of two independent p65 binding domains within TAF_{II}105 Δ C and strongly suggest that the direct interaction between TAF_{II}105 and p65/RelA plays a role in activation of anti-apoptotic genes.

5 VI. TAF_{II}105 Δ C transgenic mice

Another approach which may be taken to study the *in vivo* function of TAF_{II}105 is to generate a transgenic mouse model that expresses dominant negative mutants of TAF_{II}105 Δ C. To construct a TAF_{II}105 Δ C transgene, TAF_{II}105 cDNA encoding for the non-conserved N-terminus (amino acid 1-552) cloned in pCGN was
10 used. This is a vector that allows high expression level of a transgene. In addition, an in frame nuclear localization signal and influenza virus hemagglutinin tag (HA) were added to allow nuclear transport of the transgene product and easy detection by anti-HA antibody (Fig. 12A).

This DNA was microinjected into fertilized mouse eggs that were
15 recovered from superovulated females that had previously mated with males. At the 2-cell stage the embryos were implanted into the oviducts of pseudopregnant foster animals. Using this procedure, several animals were developed from microinjected eggs. These animals were examined for the transgene by Southern blot analysis using total genomic DNA that was prepared from tail biopsies several weeks after birth. As
20 shown in Fig. 12B, several positive animals in which the transgene has been stably integrated were obtained. The positive animals were mated with wild-type animals. After the birth of the F1 generation, the expression of the transgene mRNA and protein are determined.

To determine whether expression of a dominant negative mutant of
25 TAF_{II}105 has an effect on the transgenic animals, and particularly on the composition of the hematopoietic cell population, the health of transgenic animals of different ages may be compared to non-transgenic wild-type animals. In addition, a histological examination may be performed on spleen, thymus, lymph node, bone marrow, liver and if necessary other tissues. Furthermore, thymus and spleen organs may be
30 subjected to immunostaining analysis using antibodies directed against different types

of lymphocytes. Subsequently, a cell suspension prepared from hematopoietic tissues such as spleen, thymus and bone marrow may be subjected to FACS analysis using a panel of differentiation markers, and compared to wild-type animals. Both techniques allow the identification of cell populations which are affected by this mutant.

5 It is of particular interest to compare the phenotype of these animals with those of NF- κ B, and TNF- α ligand and receptor deficiency. If an affected population is found, the mRNA levels of some tissue/stage specific genes may be measured by Northern blot analysis and compared to those of wild-type animals. mRNAs which will be affected by hTAF_{II}105 Δ C expression are likely to be its target genes (direct or
10 indirect). Also, primary cells (B or T) from the transgenic animals may be cultured and analyzed for normal cellular processes of this particular tissue, such as transcriptional activation of certain promoters, the specific response to a certain cytokine, etc.

 If an *in vivo* effect is found, the region within the TAF_{II}105 N-terminus
15 which confers this effect upon overexpression may be determined. To this end, new transgenic mice may be generated expressing different mutants of TAF_{II}105 Δ C according to the *in vitro* binding assays and the transfection results. The phenotype of the transgenic animals may be determined in a similar manner. It is of particular interest to determine if there is a correlation between the activator binding sites and
20 the *in vivo* effect, thus linking the biochemical and genetic approaches.

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CLAIMS:

1. A DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2 or such a modified fragment, wherein said fragment or modified fragment has a dominant negative
5 effect on the normal biological activity of said TAF_{II}105 polypeptide.
2. A DNA molecule according to Claim 1 wherein said fragment is derived from the N-terminal domain of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2.
3. A DNA molecule according to Claim 2 of SEQ. ID. NO: 1 wherein
10 said nucleotide sequence encodes a TAF_{II}105 Δ C polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.
4. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a fragment of the TAF_{II}105 Δ C polypeptide having the amino acid sequence selected from the group consisting of amino acids 1-452, 1-359
15 and 443-552.
5. A DNA molecule according to Claim 1 wherein said nucleotide sequence encodes a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the TAF_{II}105 Δ C polypeptide such that the thus obtained modified fragment has a dominant negative effect on the
20 normal biological activity of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2.
6. An expression vector comprising the DNA molecule of Claim 1 and DNA sequences required for its expression.
7. A polypeptide consisting of a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2 or a modified fragment thereof, wherein said fragment or
25 modified fragment has a dominant negative effect on the normal biological activity of said TAF_{II}105 polypeptide.
8. A polypeptide according to Claim 7 which is a fragment derived from the N-terminal domain of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2.

9. A polypeptide according to Claim 8 of SEQ. ID. NO: 1, which is the TAF_{II}105 Δ C polypeptide consisting of amino acids 1 through 552 of SEQ. ID. NO: 2, or a part thereof.
10. A polypeptide according to Claim 9 which is a fragment of the
5 TAF_{II}105C polypeptide selected from the group consisting of amino acids 1-452, 1-359 and 443-552.
11. A polypeptide according to Claim 7 which is a modified fragment obtained by internal deletion, replacement or addition of one or more amino acids of the TAF_{II}105 Δ C polypeptide such that the thus obtained modified fragment
10 has a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2.
12. A DNA molecule directing expression of an antisense RNA sequence to SEQ. ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.
13. A pharmaceutical composition for inducing an apoptotic process in
15 pathological cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:
- (a) a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2 or such a fragment modified by internal deletion, replacement or addition of one or
20 more amino acids, wherein the fragment or the modified fragment has a dominant negative effect on the normal biological activity of the TAF_{II}105 polypeptide;
 - (b) an inhibitor or antagonist of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2;
 - (c) a DNA sequence encoding the fragment or modified fragment of
25 (a); and
 - (d) a DNA sequence directing expression of an antisense RNA sequence to SEQ. ID. NO.: 1, or a part thereof, and which is capable of inhibiting its expression *in vivo*.

14. A pharmaceutical composition according to Claim 13 wherein the active agent (a) is a polypeptide consisting of a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2 or a modified fragment thereof, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said TAF_{II}105 polypeptide.
15. A pharmaceutical composition according to Claim 13 wherein the active agent (c) is a DNA molecule comprising a nucleotide sequence encoding a fragment of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2 or such a modified fragment, wherein said fragment or modified fragment has a dominant negative effect on the normal biological activity of said TAF_{II}105 polypeptide.
16. A pharmaceutical composition according to Claim 13 for the treatment of cancer.
17. A method for promotion of apoptosis comprising administering to a subject in need, a pharmaceutical composition according to Claim 13.
18. A pharmaceutical composition for treatment of pathological apoptosis of cells comprising a pharmaceutically acceptable carrier and an active agent selected from the group consisting of:
- (e) a DNA molecule comprising the DNA sequence of SEQ. ID. NO: 1;
 - (f) a DNA molecule consisting of a DNA sequence encoding the TAF_{II}105 polypeptide of SEQ. ID. NO: 2;
 - (g) a modified DNA sequence of (a) or (b) in which one or more nucleotide triplets have been added, deleted or replaced, wherein the polypeptide encoded by the modified DNA sequence retains the normal biological activity of the TAF_{II}105 polypeptide of SEQ. ID. NO: 2;
 - (h) the TAF_{II}105 polypeptide of SEQ. ID. NO: 2; and
 - (i) a modified polypeptide of (d) in which one or more amino acids have been added, deleted or replaced, wherein the modified polypeptide retains the normal biological activity of the TAF_{II}105 polypeptide encoded by SEQ. ID. NO: 2.

19. A pharmaceutical composition according to Claim 18 for the treatment of autoimmune diseases, inflammatory processes and viral or bacterial infections.

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GGGACCCTGGTGACCAAAGTGGCTCCGGTCAGCGCCCCTCCTAAAGTCAGCA
GCGGCCCTAGGCTGCCTGCTCCTCAGATAGTCGCCGTGAAAGCCCCCAACAC
CACGACAATCCAGTTTTCTGCTAATTTGCAGCTTCCTCCAGGAACCGTTTTGA
TTAAAAGTAACAGTGGTCCGTTGATGTTGGTATCTCCTCAGCAAACCTGTAACA
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CCTCAAACAGTCAAAATCTGTACAGTGCCGAACCTCTAGCTCACAATTAATCA
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CCTTCAAGTTTGGGAGCATCATCCACTCCTTCAAATGAGCCCAATCTTAAAGC
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GTGAAGAAATGCAAGAACTTCCTTGCAATGTTAATAAAACTAGCATGTAGTG
GATCACAGTCCCCTGAAATGGGGCAAATGTGAAGAAGCTGGTGGAACAACT
TTTGATGCAAAAATCGAAGCAGAAGAATTTACTAGGAAACTGTATGTTGAA
CTCAAGTCTTCACCTCAGCCTCACCTGGTTCCTTTTCTTAAGAAAAGCGTGGT
TGCCTTACGACAACCTTCTGCCTAACTCCCAGAGCTTCATCCAGCAATGTGTTT
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TCTCCTGTGGTGACAACCTACAGTGTCTCAAGCCAGTCTGAAAAGTCAATTAT
TGTTTCTGGAGCAACAGCACCCAGAAGCTGTGTGAGTGCAAACTTTGAACCCA
CTTGCTGGTCCAGTGGGAGCAAAAGCTGGAGTTGTGACACTTCATTCTGTGG
GCCCAACTGCTGCAACAGGAGGAACAACAGCTGGAACCTGGTTTGCTTCAGAC
TTCAAAACCACTTGTGACATCTGTGGCAAACACAGTGACCACGGTCTCACTG
CAACCTGAAAAGCCAGTTGTCTCTGGAACAGCAGTAACACTGTCCCTTCCAG
CAGTAACTTTTGGAGAACTTCAGGTGCAGCTATTTGTCTTCCATCTGTGAAA
CCTGTTGTTTCTTCTGCTGGGACCACATCTGCAAGCCTGTTATTGGGACTCC
AGTTCAAATCAAACCTTGCCCAGCCGGGCCCTGTCTTTTACAACCAAGCTGGG
ATTCCAACAGGCAGTTCAAGCAAGCAACTATTCTCATTGTTTACGTAAGTTCA
GCAGCCTTCAGGAGGCAATGAAAAACAAGTGACCACAATTTACATTCTCTCA
ACATTGACCATTACAGAAATGTGGACAGAAGACGATGCCAGTGAACACCATAA
TACCTACTAGTCAGTTTTCTCCAGCTTCCATTCTAAAGCAAATTACTCTGCCT
GGAAATAAAATTCTGTCACTTCAAGCATCTCCTACTCAGAAAAATAGAATAA
AAGAGAATGTAACATCATGCTTCCGAGATGAGGATGACATCAATGATGTGAC
TTCTATGGCAGGGGTCAACCTTAATGAAGAAAATGCCTGCATCTTAGCAACA
AACTCTGAATTGGTTGGCACACTCATTAGTCATGTAAAGATGAACCATTTCT
TTTTATTGGAGCTCTACAAAAGAGAATCTTAGACATTGGTAAAAAGCATGAC
ATTACAGAACTTAACCTCTGATGCTGTGAACCTTGATCTCCCAAGCAACACAGG
AACGACTACGAGGCCTTCTAGAAAAACTGACTGCAATTGCTCAGCATCGAAT
GACTACTTACAAGGCAAGTGAAAATTACATCCTGTGTAGTGATACCAGGTCA
CAGCTCAAATTTCTTGAAAAGCTGGATCAATTGGAGAAGCAGAGAAAGGATT
TGGAAGAAAGAGAAATGTTACTTAAGGCAGCCAAGAGTCGTTCTAATAAAGA
AGATCCAGAACAGCTGAGATTAAAGCAGAAAGCCAAAGAGTTACAGCAATT
GGAACCTTGACAGATACAGCATAGAGACGCTAATCTCACAGCTCTTGACAGCT
ATTGGACCAAGGAAGAAGAGACCACTAGAATCTGGAATTGAGGGCTTAAAA
GACAACCTTCTTGCTTCTGGGACATCCAGCCTGACAGCCACCAACAGTTGC
ATCGTCCAAGAATCACGAGAATCTGCCTCAGGGACTTGATATTTTGTATGGA
ACAGGAACGGGAGATGAAGTATTCTCGAGCTCTATACCTGGCCCTTCTG

Fig. 1

2/13

AAGTGACCACTCCACTCTTCCATCCACATCCTTGCTATTTACTGCCAAAGAAG
ACACAAAGCATTGTTGCACTGTCCTGAAATTTCAATTTCTGGAAAATAACACC
AACATGAAAGAGCATTGTTTACGATTAGAACTTTATTA ACTCTTACCTAT

Fig. 1 (Con't)

3/13

GTLVTKVAPVSAPPKVSSGPRLPAPQIVAVKAPNTTTIQFPANLQLPPGTVLIKSN
GPLMLVSPQQTIVTRAETTSNITSRPAVPANPQTVKICTVPNSSSQLIKKVAVTPVK
KLAQIGTTVVTTVPKPSSVQSVAVPTSVVTVTPGKPLNTVTTLPSSLGASSTPSN
EPNLKAENSAAVQINLSPTMLENVKKCKNFLAMLIKACSGSQSPMGQNVKKL
VEQLLDAKIEAEFTRKLYVELKSSPQPHLVPFLKKSVALRQLLPNSQSFIQQCV
QQTSSDMVIATCTTTVTTSPVVTTTVSSSQSEKSIIVSGATAPRTVSVQTLNPLAGP
VGAKAGVVTLHSVGPATAATGGTTAGTGLLQTSKPLVTSVANTVTTVSLQPEKPV
VSGTAVTSLPAVTFGETSGAAICLPVSKPVVSFCWDHICKPVIGTPVQIKLAQPG
PVLSQPAGIPTGSSSKQLFSLFHVVQQPSGGNEKQVTTISHSSTLTIQKCGQKTMP
VNTIPTSQFPPASILKQITLPGNKILSLQASPTQKNRIKENVTSCFRDEDDINDVTS
MAGVNLNEENACILATNSELVGTLIQSCKDEPFLFIGALQKRILDIGKKHDITELNS
DAVNLSQATQERLRGLLEKLTALAQHRMTTYKASENYILCSDTRSOLKFLEKLD
QLEKQRKDLEREMLLKAAKSRSNKEDPEQLRLKQKAKELQQLELAQIQHRDAN
LTALAAIGPRKKRPLESGIEGLKDNLLASGTSSLTATKQLHRPRITRICLRDLIFCM
EQEREMKYSRALYLALLKZPLHSSIHLAIYCQRRHKALLHCPEISISGKZHHERA
LFTIRTLTLTY

Fig. 2

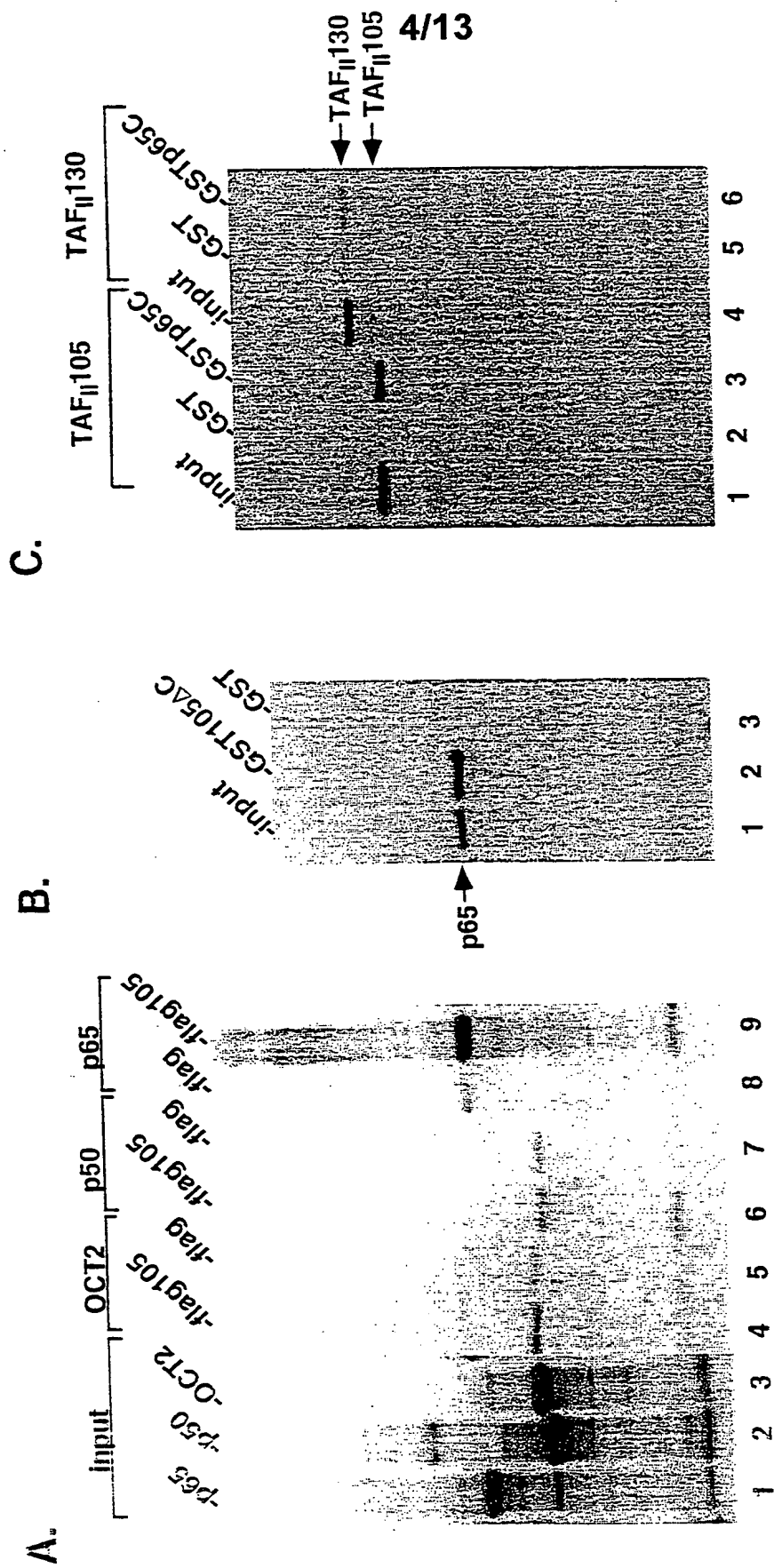


Fig. 3

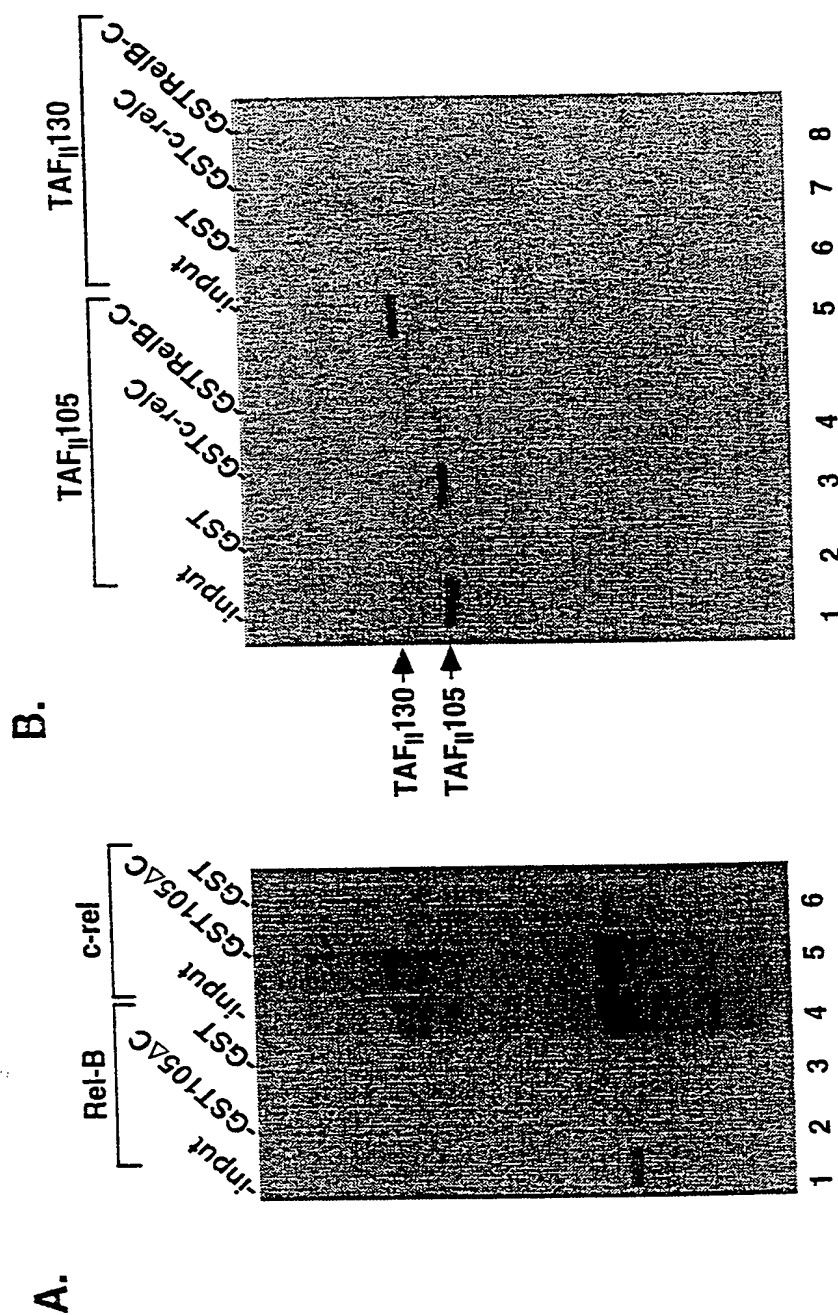


Fig. 4

6/13

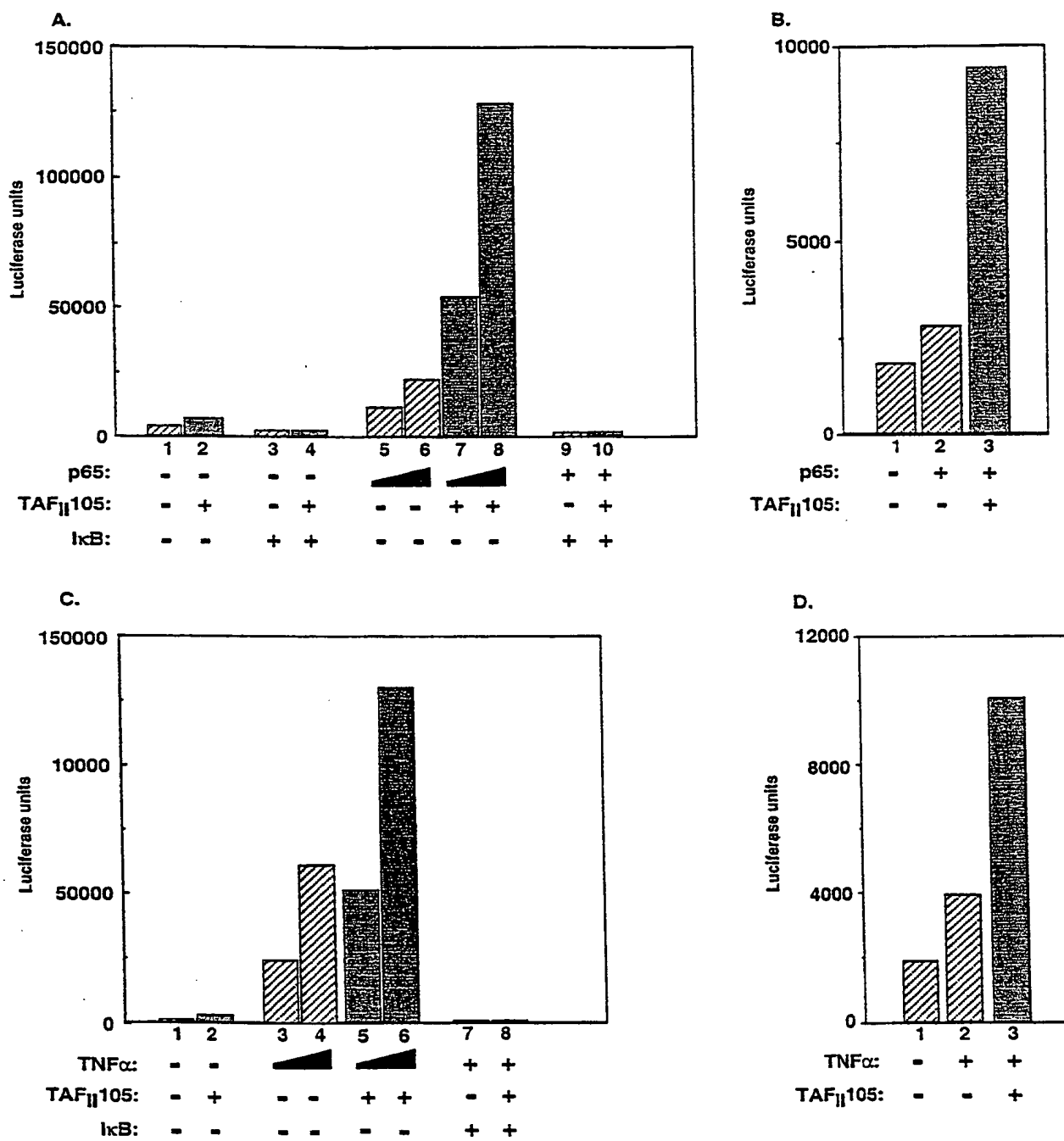
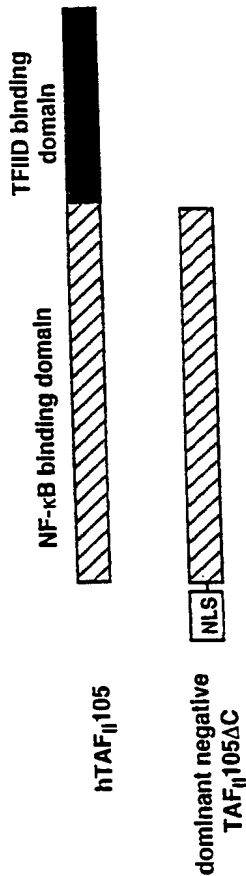
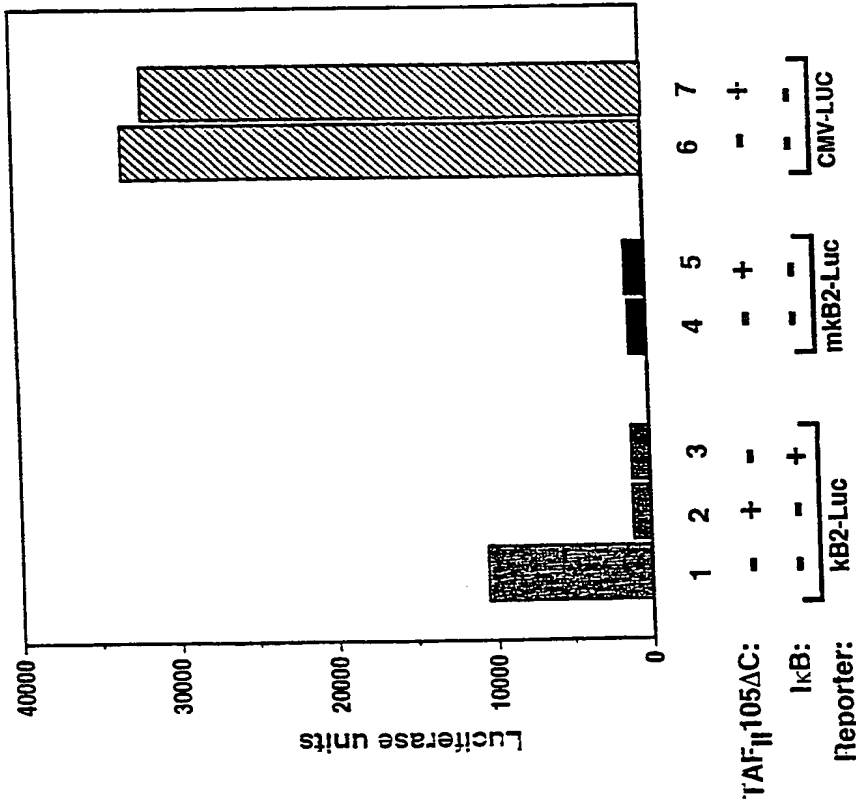


Fig. 5

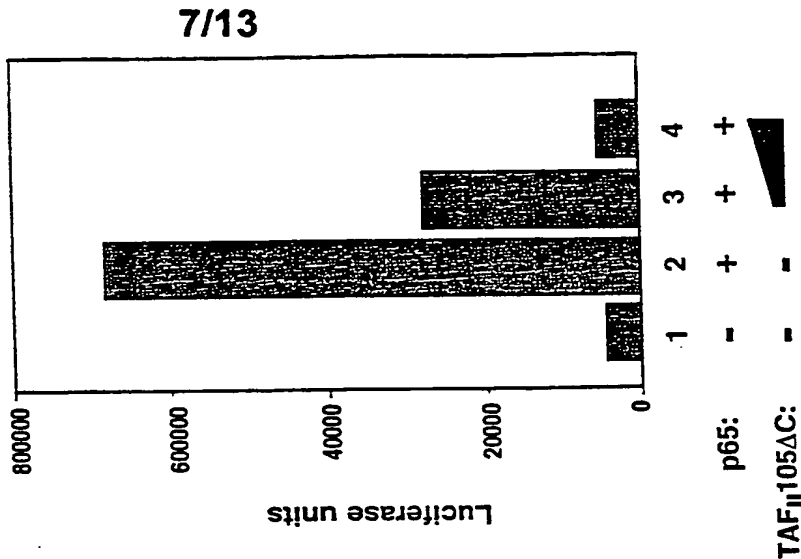
A.



B.



C.

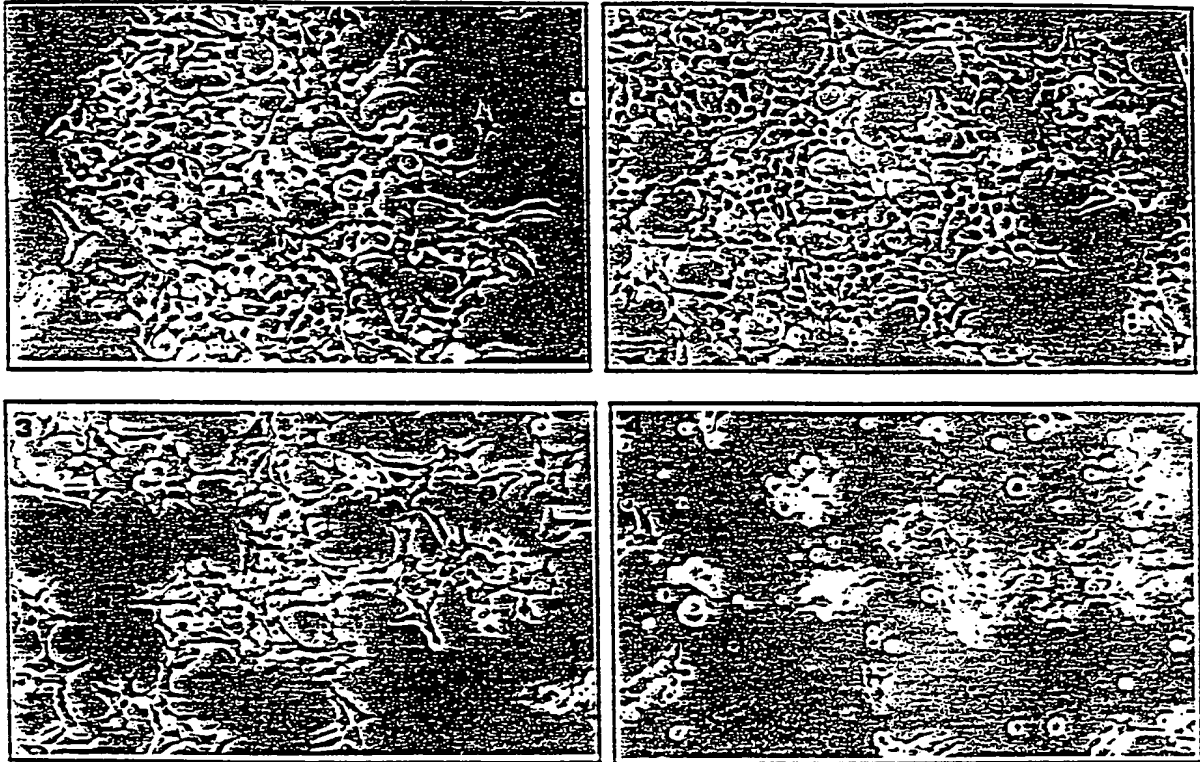


7/13

Fig. 6

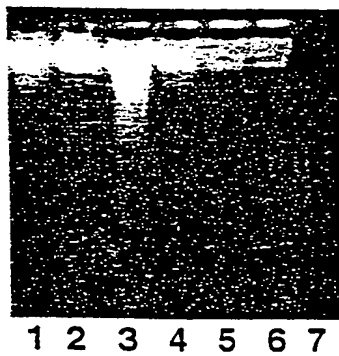
A.

8/13



B.

TAF _{II} 105	-	-	-	-	+	+	
TAF _{II} 105ΔC	-	-	+	+	-	-	
TNFα	+	-	+	-	+	-	M



C.

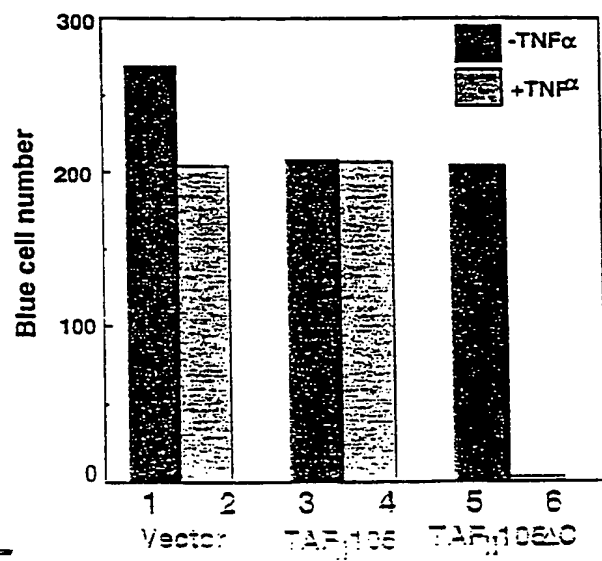


Fig. 7

9/13

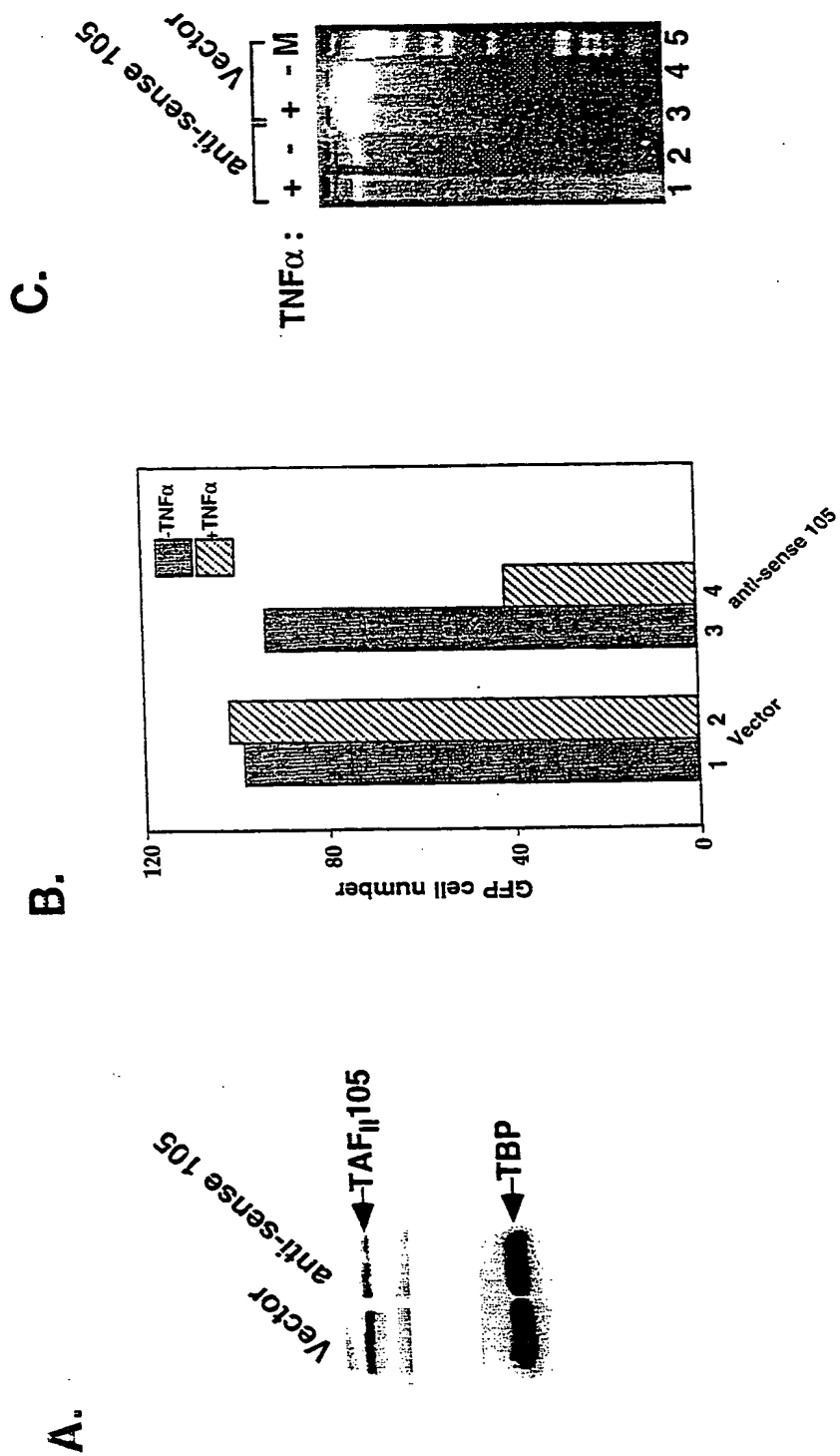
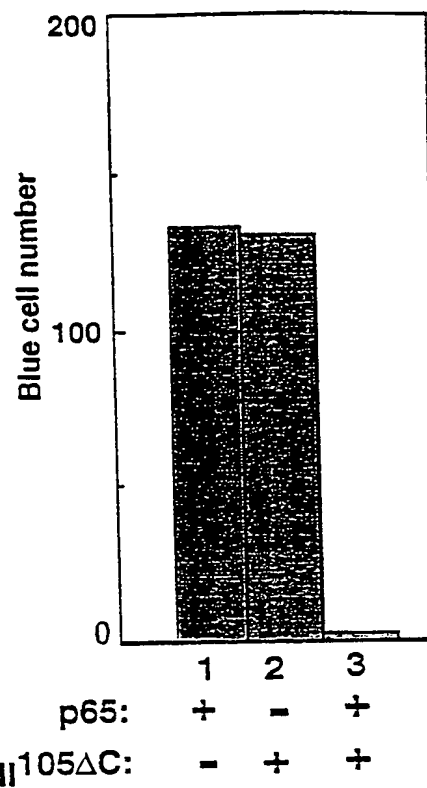
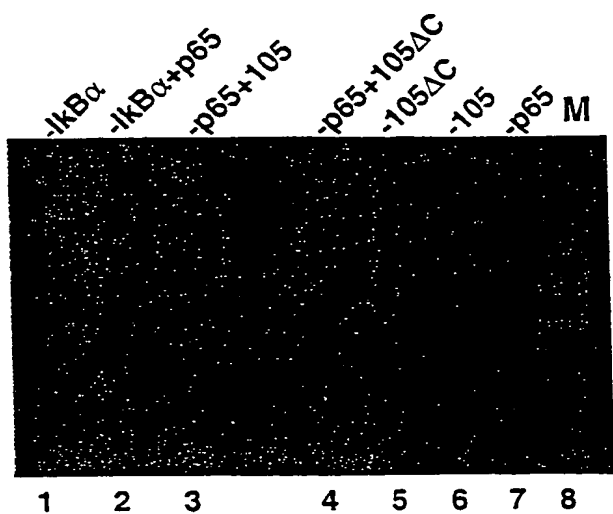


Fig. 8

A.

10/13

C.



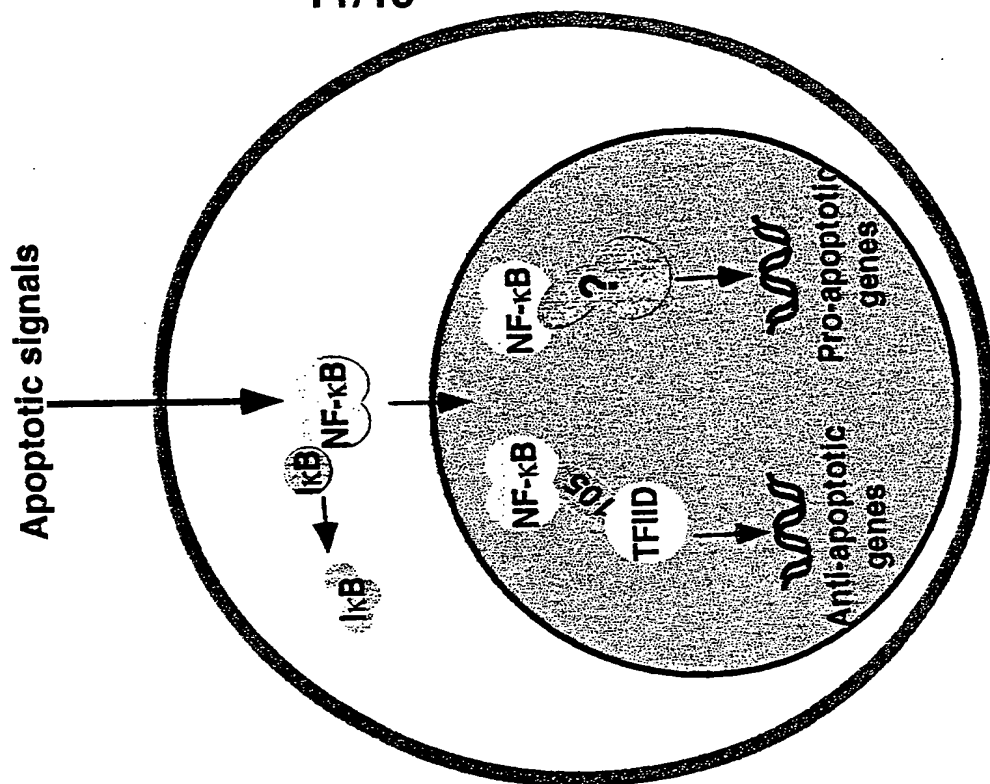
B.



Fig. 9

11/13

B.



A.

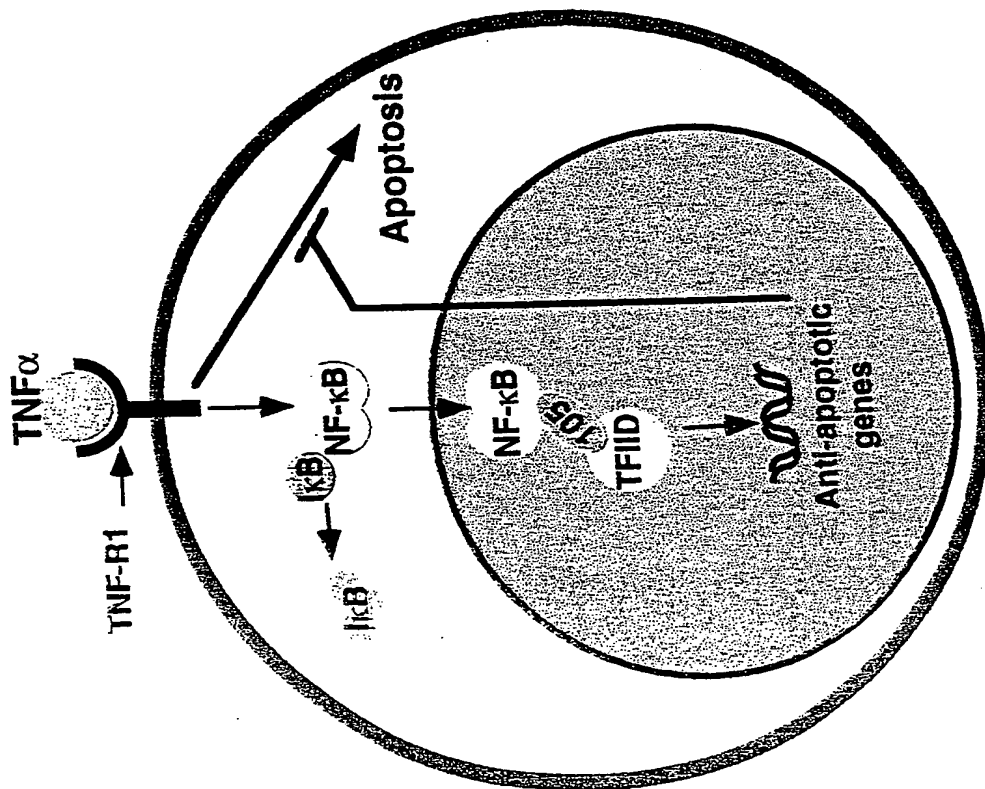


Fig. 10

12/13


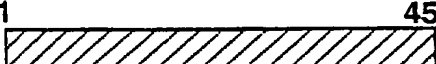
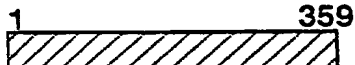
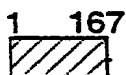
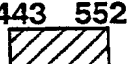
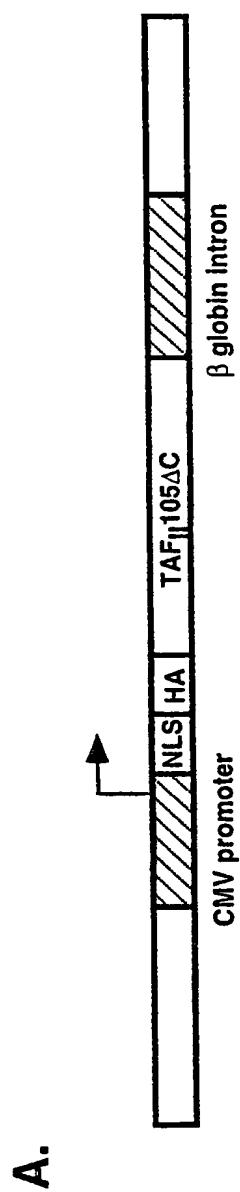
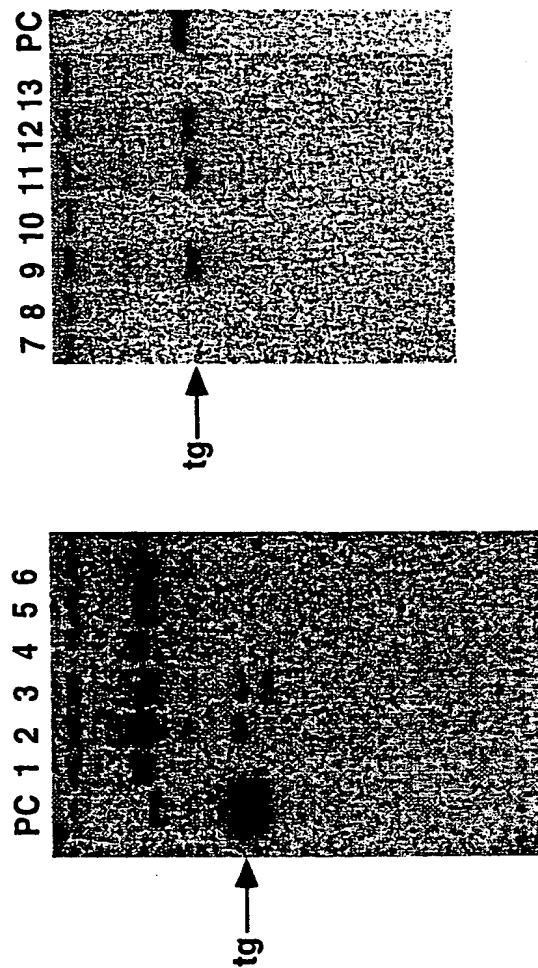
	<u>p65 binding</u>	<u>Cell death</u>
	+++	+++
	+	+
	+	+
	-	-
	++	++

Fig. 11

13/13

**B.****Fig. 12**

TENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
 United States Patent and Trademark
 Office
 Box PCT
 Washington, D.C.20231
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

31 March 2000 (31.03.00)

International application No.

PCT/IL99/00464

Applicant's or agent's file reference

119487.7 MM

International filing date (day/month/year)

25 August 1999 (25.08.99)

Priority date (day/month/year)

27 August 1998 (27.08.98)

Applicant

DIKSTEIN, Rivka et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

28 February 2000 (28.02.00)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Pascal Piriou

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00464

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/47 C12N15/11 A61K38/17 A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DIKSTEIN R ET AL: "Human TAFII 105 is a cell type-specific TFIID subunit related to hTAFII130." CELL, (1996 OCT 4) 87 (1) 137-46. , XP000857418 cited in the application the whole document	1-19
Y	US 5 710 025 A (DIKSTEIN RIVKA ET AL) 20 January 1998 (1998-01-20) cited in the application the whole document	1-19

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

27 January 2000

Date of mailing of the international search report

03/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YAMIT-HEZI, AYALA ET AL: "TAFII105 mediates activation of anti-apoptotic genes by NF - kappaB." EMBO (EUROPEAN MOLECULAR BIOLOGY ORGANIZATION) JOURNAL, (SEPT. 1, 1998) VOL. 17, NO. 17, PP. 5161-5169. , XP000857189 the whole document ---	1-19
Y	N. TANESE ET AL.: "Molecular cloning and analysis of two subunits of the human TFIID complex: hTAFII130 and hTAFII100." PROC. NATL. ACAD. SCI. USA, vol. 93, November 1996 (1996-11), pages 13611-13616, XP000867132 the whole document ---	1-19
Y	WO 94 17087 A (UNIV CALIFORNIA) 4 August 1994 (1994-08-04) the whole document ---	1-19
A	DIKSTEIN, RIVKA ET AL: "TAF-II250 is a bipartite protein kinase that phosphorylates the basal transcription factor RAP74." CELL, (1996) VOL. 84, NO. 5, PP. 781-790. , XP000857186 the whole document ---	
A	B F PUGH AND R TIJAN: "Mechanism of transcriptional activation by SP1: evidence for coactivators" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 61, page 1187-1197 XP002079600 ISSN: 0092-8674 cited in the application the whole document ---	
A	HORIKOSHI M ET AL: "TRANSCRIPTION FACTOR ATF INTERACTS WITH THE TATA FACTOR TO FACILITATE ESTABLISHMENT OF A PREINITIATION COMPLEX" CELL,US,CELL PRESS, CAMBRIDGE, NA, vol. 54, page 1033-1042 XP000857420 ISSN: 0092-8674 cited in the application the whole document ---	
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00464

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HOFFMANN A ET AL: "CONSIDERATIONS OF TRANSCRIPTIONAL CONTROL MECHANISMS: DO TFIID-CORE PROMOTER COMPLEXES RECAPITULATE NUCLEOSOME-LIKE FUNCTIONS?" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,US,NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 94, page 8928-8935 XP000857421 ISSN: 0027-8424 cited in the application the whole document -----</p>	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 99/00464

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5710025	A	20-01-1998	NONE	
WO 9417087	A	04-08-1994	AU 682340 B	02-10-1997
			AU 6131194 A	15-08-1994
			CA 2154882 A	04-08-1994
			EP 0681585 A	15-11-1995
			JP 8509119 T	01-10-1996
			US 5534410 A	09-07-1996
			US 5637686 A	10-06-1997

PATENT COOPERATION TREATY

PCT

REC'D 05 DEC 2000

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



15

Applicant's or agent's file reference 119487.7 MM	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/IL99/00464	International filing date (day/month/year) 25/08/1999	Priority date (day/month/year) 27/08/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
 - ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:
 - I ☒ Basis of the report
 - II ☐ Priority
 - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI ☐ Certain documents cited
 - VII ☐ Certain defects in the international application
 - VIII ☒ Certain observations on the international application

Date of submission of the demand 28/02/2000	Date of completion of this report 30.11.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Renggli-Zulliger, N Telephone No. +49 89 2399 7482 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL99/00464

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).):*

Description, pages:

1-25 as originally filed

Claims, No.:

1-19 as originally filed

Drawings, sheets:

1/13-13/13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL99/00464

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 13-19.

because:

☒ the said international application, or the said claims Nos. 13-19 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 4, 10, 14-19

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL99/00464

	No:	Claims	1-3, 5-9, 11-13
Inventive step (IS)	Yes:	Claims	4, 10, 14-17
	No:	Claims	1-3, 5-9, 11-13, 18, 19
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	-

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 13-19 as far as *in vivo* treatments are concerned relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: DIKSTEIN R ET AL: 'Human TAFII 105 is a cell type-specific TFIID subunit related to hTAFII130.' CELL, (1996 OCT 4) 87 (1) 137-46, cited in the application.

D2: US-A-5 710 025 (DIKSTEIN RIVKA ET AL) 20 January 1998, cited in the application .

Novelty (Article 33(1) and (2) PCT) and Inventive step (Article 33(1) and (3) PCT)

1) D1 or D2 disclose the nucleic acid sequence and amino acid sequence of human TAF_{II}105 as mentioned in the present application (page 2, lines 12-15). The nucleic acid sequence is 100% identical to sequence ID n°1(2556bp) over a 2556 bp overlap and 100% identical to the encoded protein over 801 aa (Seq. ID n°2: 852aa) (see remarks under RE Item VIII of this IPER).

2) D1 discloses a nucleotide sequence that encodes a polypeptide of 801 aa that is identical to the sequence ID n°2 and can be designated as a fragment or a modified fragment of TAF_{II}105. This fragment has a dominant negative effect on the normal

biological activity of said TAF₁₁105 polypeptide, as it contains amino acids 1-552 that are involved in the above mentioned function. Furthermore, the biological function i.e. the activation of anti-apoptotic genes by NF- κ B or dominant negative effect is considered as an inherent feature of the protein, therefore, the subject-matter of claim 1, 2, 7 and 8 is not novel.

3) Furthermore, fragments derived from the N-terminal domain of TAF₁₁105 in which 5-10 N-terminal residues are missing (page 141, first column, lines 10-15) or a fragment consisting in the N-terminal portion of the protein expressed in *E.coli* (page 142, first column, first line) are also disclosed. These fragments can be designated as a part of TAF₁₁105 Δ C, or modified fragments by deletion. Since the polynucleotide sequence of the full-length TAF₁₁105 is disclosed, the DNA molecules encoding such fragments is also disclosed. Therefore, the subject-matter of claim 3, 5, 9 and 11 is not novel.

4) D2 discloses incomplete translates of the Seq. ID n°1 and DNA molecules that encodes deletion mutants of Seq. ID n°2 that comprises at least 20 amino acids of Seq. ID n°2 and preferably the C-terminus portion from aa 547-801 or N-terminus part from aa 1-546 of Seq. ID n°2 (column 2, lines 15-31) as well as the corresponding polypeptide fragments. Nucleic fragments comprising Seq. ID n°1 or fragments thereof are also disclosed (column 4, lines 5-7). In this case, the dominant negative effect on normal biological activity of said TAF₁₁105 polypeptide is also considered as an inherent feature of the protein that does not render the subject-matter of claims 1-3, 5, 7-9 and 11 novel over the fragments disclosed in D2.

5) D1 and D2 also disclose an expression vector containing the fragments of the N-terminal portion of sequence ID n°1 expressed in *E.coli* (D1: page 142, first column, first line) or in expression systems, in general (D2: column 3, lines 30-43) or in baculovirus in particular (D2: column 9, last paragraph). Therefore, the subject-matter of claim 6 is not novel.

6) RNase protection assays were performed with specific anti-sense RNA probes for TAF₁₁105 that may also inhibit its expression *in vivo* (D1: page 145, second column, "RNA Analysis", lines 7-12; D2: column 8, lines 9-14). Therefore, the subject-matter of claim 12 is not novel.

7) D2 discloses a composition for use in diagnosis, therapy and pharmaceutical development comprising hTAF_{II}105-specific receptors or antibodies (inhibitor or antagonist) or binding agents that modulate the TAF_{II}105 function (column 3, lines 6-29, column 4, lines 31-50). Therefore, the subject-matter of claim 13 is not novel.

8) D2 is considered as the closest prior art document that discloses the protein and nucleic acid sequences of TAF_{II}105 as well as fragment thereof.

The difference between the present application and D2 is the provision of a fragment able to modulate anti-apoptotic genes through NF-κB.

The problem to be solved is therefore the provision of modulator of anti-apoptosis genes by NF-κB.

The solution is the use of TAF_{II}105ΔC fragments 1-452, 1-369 and 443-552.

The use of these specific fragments was not suggested as well as their role in as dominant negative regulator of apoptosis could not be derived from the cited prior art. Therefore, the subject-matter of claims 4, 10, 14-17 is novel and inventive.

9) However, the subject-matter of claims 18-19 encompasses fragments of the protein such as for example TAF_{II}105ΔN, for which no anti-apoptotic function is demonstrated but that retains a part of the normal biological activity of TFIID i.e. the TFIID binding domain (see also RE Item VIII, point 2).

The subject-matter of the claims should correspond to and be justified by the technical contribution to the art, and therefore, obvious subject-matter should be excluded. The use of fragments of a known proteins in a pharmaceutical composition or a method of treatment with no particular effect is obvious and does not solve the problem posed.

It is noted also that the part 13(d) does not involve an inventive step, as the use of antisense RNA in a therapy to inhibit the expression of a known protein *in vivo* is obvious to the person skilled in the art and is part of the common general knowledge.

Therefore, the subject-matter of claims 13, 18-19 does not involve an inventive step.

Industrial applicability (Article 33(1) and (4) PCT)

10) For the assessment of the present claims 13-17 on the question whether they are

industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

- 1) The terms "fragments" used in claims 1, 2, 4, 5, 7, 8, 10, 11, 13-15 or "part" used in claims 3, 9, 12, 13 are not clear in the sense of Article 6 PCT, because the size of such portions or parts is not defined rendering the limits of the claims undefined too.
- 2) The term "biological activity" in claims 1, 2, 7, 11, 13, 14, 15, 18, 19 is not clear under Article 6 PCT, because what is referred to is not unambiguously derivable from the claim wording alone. Furthermore, a "normal" biological activity is a relative term that is open to interpretation, because it is not clear if s the TFIID binding domain and the NF- κ B binding domain are necessary or if only one is sufficient as for TAF_{II}105 Δ C (see claim 5, for example), then only the TFIID binding domain (C-terminal part of TAF_{II}105) may be also sufficient.
- 3) The term "modified" in claims 1, 5, 7, 11, 13, 18 is not clear (Article 6 PCT), because the nature and the extent of such modifications is not clear limited, and can encompass subject-matter that is not structurally related.

Additional remarks:

- 4) There is a discrepancy between the description and the sequence listings. The sequence cited as the reference for TAF_{II}105 is the one disclosed by the applicants themselves (D1, D2) (page 2, lines 12-15) as well as in the priority document. This sequence is 801aa long and the last amino acid is a lysine, whereas in the present application Seq. ID n°2 has an additional 51 amino acids. Curiously, although the reading

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IL99/00464

frame is the same, the stop codon of sequence ID n°1 that is 100% identical to the one of D1 or D2 over the full-length, becomes a Glx in the present application, the next amino acids can be normally deduced from Seq. ID n°1 in the same reading frame. This renders the sequence of the protein unclear. It is noted that the priority document (26 August 1998) pertaining to the present application discloses only the amino acids 1-801.